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# Emulsifying, rheological and physicochemical properties of exopolysaccharide produced by *Bifidobacterium longum* subsp. *infantis* CCUG 52486 and *Bifidobacterium infantis* NCIMB 702205

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ABSTRACT

The rheological, emulsification and certain physicochemical properties of purified exopolysaccharides (EPS) of *Bifidobacterium longum* subsp. *infantis* CCUG 52486 and *Bifidobacterium infantis* NCIMB 702205 were studied and compared with those of guar gum and xanthan gum. The two strains were grown in skim milk supplemented with 1.5% (w/v) casein hydrolysate at 37 °C for 24 h; they both produced heteropolysaccharides with different molecular mass and composition. The carbohydrate content of both polymers was more than 92% and no protein was detected. The EPS of *B. longum* subsp. *infantis* CCUG 52486 showed highly branched entangled porous structure under scanning electron microscopy. Higher intrinsic viscosity was observed for the EPS of *B. longum* subsp. *infantis* CCUG 52486 compared to the EPS of *B. infantis* NCIMB 702205 and guar gum. Both polymers showed pseudoplastic non-Newtonian fluid behaviour in an aqueous solution. The EPS of *B. infantis* NCIMB 702205 and *B. longum* subsp. *infantis* CCUG 52486 produced more stable emulsions with orange oil, sunflower seed oil, coconut oil and xylene compared to guar and xanthan gum. The EPS of *B. longum* subsp. *infantis* CCUG 52486 is the most promising one for applications in the food industry, as it had higher intrinsic viscosity, higher apparent viscosity in aqueous solution, porous dense entangled structure and good emulsification activity.



## 1. Introduction

Many species of bacteria are reported to produce exopolysaccharides (EPS) which are either loosely attached to the cell surface or completely excreted to the environment as slime. EPS produced by different bacteria have been reported to be used in a wide range of applications including food products, pharmaceuticals, bioemulsifiers, bioflocculants and chemical products (Wang, Ahmed, Feng, Li, & Song, 2008). There is high demand for EPS from different species of bacteria as newly emerging industrially important biopolymers, due to their generally recognised as safe (GRAS) status, environmentally friendly production and higher potential activity at low concentration compared to commercially available polymers (Gutiérrez, Leo, Walker, & Green, 2009; Kanmani et al., 2011). In addition, bacterial EPS have been shown to be effective as immunomodulatory, immunostimulatory, antitumor, anti-inflammatory, and antioxidant agents (Liu et al., 2010).

Bacterial polysaccharides have some advantages over plant or animal hydrocolloids in the food industry; the quantities of hydrocolloids available from plant and animal origin are not sufficient to fulfil the demand (Laws, Gu, & Marshall, 2001), and sometimes they do not produce the desired rheological properties (Saija, Welman, & Bennett, 2010). In addition, there is no assurance of constant price and supply of plant hydrocolloids due to drought and other environmental factors existing in the areas where these plants are grown (Iyer, Mody, & Jha, 2006). Furthermore, religious and vegetarian lifestyle choices restrict some consumers from eating foods (yoghurt, ice cream and whipped desserts) containing animal-based hydrocolloids; gelatine is an example (Karim & Bhat, 2008).

The common EPS producing bacterial species used in food applications include streptococci (Qin et al., 2011; Säwén, Huttunen, Zhang, Yang, & Widmalm, 2010), lactobacilli (Rodríguez-Carvajal et al., 2008; Wang et al., 2010), lactococci (Ayala-Hernández, Hassan, Goff, de Orduña, & Corredig, 2008; Costa et al., 2010) and bifibobacteria (Prasanna, Grandison, & Charalampopoulos, 2012b; Ruas-Madiedo et al., 2007). Most microbial polysaccharides show higher water solubility compared to plant gums, which is a very important characteristic in various food applications (Kumar Ganesh, Joo, Choi, Koo, & Chang, 2004). In addition, EPS produced

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by various species of bacteria have been shown to have higher viscosifying, thickening, stabilising, gelling and emulsifying activities than some other commercially used polymers such as guar gum, locust bean gum and gum arabic (Freitas et al., 2009; Jindal, Singh, & Khattar, 2011; Kanmani et al., 2011; Wang et al., 2008). Furthermore, EPS produced by different bacterial species have been shown to have stable rheological and emulsifying properties over wide ranges of temperature, pH and ionic strength, which ensure their application in various food applications under different conditions (Kumar & Mody, 2009).

Bifidobacteria are an important group of probiotic cultures commonly used in various food applications. In a previous study we have reported on new EPS producing *Bifidobacterium longum* subsp. *infantis* CCUG 52486 and *Bifidobacterium infantis* NCIMB 702205 when they were grown in skim milk (Prasanna et al., 2012b). The physicochemical, rheological and emulsifying properties of purified EPS from many species of lactic acid bacteria (LAB) have been reported. In addition, the EPS from LAB have been shown to have potential application in food products, including dairy products, by improving their rheological and textural properties (Garai-Ibabe et al., 2010). However, so far there is little information on the physicochemical properties, rheological and emulsifying properties of purified EPS from *Bifidobacterium* strains and their use in various food applications. In this study we report on certain physicochemical, rheological and emulsifying properties of EPS produced by *B. longum* subsp. *infantis* CCUG 52486 and *B. infantis* NCIMB 702205 and evaluate their potential in food applications.

## 2. Materials and methods

### 2.1. Microorganisms and culture conditions

*B. longum* subsp. *infantis* CCUG 52486 and *B. infantis* NCIMB 702205 were acquired from the culture collection of the University of Göteborg in Sweden and the UK National Collection of Industrial, Food and Marine Bacteria (NCIMB), respectively. These strains were maintained at −80 °C in trypticase phytone yeast (TPY) extract medium containing 15% (v/v) glycerol. They were activated from their frozen form by propagation in Wilkins–Chalgren anaerobe agar (WC) (Oxoid, Hampshire, UK) under anaerobic conditions at 37 °C for 72 h. Thereafter, two successive cultures of bacteria were carried out in 10 mL of TPY broth under anaerobic condition for 24 h, using an inoculum volume of 1% (v/v). Bacterial cells were harvested after the second growth cycle by centrifugation at 10,000 × *g* for 15 min, at 4 °C. The pellet was washed with sterile phosphate buffered saline (PBS) (Oxoid, UK), and then resuspended in 10 mL of commercial pasteurised skim milk to prepare the inoculum.

The milk fermentations were carried out in glass bottles (1000 mL) with screw caps. Commercial pasteurised skim milk (800 mL) was supplemented with 1.5% (w/v) of casein hydrolysate (CH) (Sigma–Aldrich, Dorset, England, UK) and heat treated at 85 °C for 30 min. It was then cooled to 37 °C and inoculated with 1% (v/v) of the inoculum. The fermentations were carried out at 37 °C, for 24 h.

### 2.2. Isolation and purification of EPS

The EPS were isolated and purified by using the method of Prasanna, Grandison, and Charalampopoulos (2012a). In brief, the EPS of the fermented milk samples were isolated *via* trichloroacetic acid treatment followed by ethanol precipitation and subsequent dialysis and lyophilisation.

### 2.3. Physicochemical characterisation of EPS

#### 2.3.1. EPS concentration, molecular mass and monosaccharide composition

The freeze dried samples were dissolved in ultrapure water and analysed by gel permeation chromatography (GPC) to determine the yield and molecular mass of the isolated EPS as described previously (Prasanna et al., 2012b). The monosaccharide composition of two EPS was determined using the method described previously by Prasanna et al. (2012b).

#### 2.3.2. Chemical analysis of EPS

The total carbohydrate content of the purified EPS was analysed using the method described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956). The total protein content was determined using the bicinchoninic acid (BCA) assay kit (Sigma–Aldrich, UK) with bovine serum albumin (BSA) as the standard. The moisture content of lyophilised EPS samples was determined as described earlier by Vijayendra, Palanivel, Mahadevamma, and Tharanathan (2008).

### 2.4. Determination of intrinsic viscosity

The intrinsic viscosity ($\eta$) of EPS and gums was determined as described by Freitas et al. (2011) and Shene, Canquil, Bravo, and Rubilar (2008). Dilute solutions of EPS, xanthan gum (Sigma–Aldrich, UK) and guar gum (Sigma–Aldrich, UK) (0.01–0.1%, w/v) were prepared with deionised water. Viscosity measurements were carried out using an Ubbelohde capillary viscometer (1619/03-C, Rheotek, UK) at 25 °C. Three independent measurements were made for each sample. The relative viscosity ($\eta_{rel} = t/t_0$), specific viscosity ($\eta_{sp} = \eta_{rel} - 1$) and reduced viscosity ($\eta_{red} = \eta_{sp}/C$) were determined; $t$ and $t_0$ are the flow time of solution and water, respectively; $C$ is the concentration of particular solution. The data were fitted to the Huggins and Kraemer equations given below and the intrinsic viscosity ($\eta$) of the two EPS, guar gum and xanthan gum were determined by extrapolating the two linear functions to zero concentration in order to obtain the average values at the intercept:

$$\frac{\eta_{sp}}{C} = [\eta] + k_H[\eta]^2 C \quad \text{(Huggins equation)}$$

$$\frac{\ln(\eta_{rel})}{C} = [\eta] + k_k[\eta]^2 C \quad \text{(Kramer equation)}$$

where $k_H$ and $k_k$ are the Huggins and Kraemer coefficients.

### 2.5. Rheological properties in aqueous solution

The rheological behaviour of EPS solutions, guar gum and xanthan gum was studied with a Bohlin C-VOR (Malvern Instruments Ltd., Malvern, UK) controlled strain rheometer, with a parallel plate geometry (PP40; 40-mm diameter and 1-mm gap setting). For this, the lyophilised EPS or gum (1%, w/v) was dissolved in distilled water. All measurements were carried out at 20 °C. A shear ramp was carried out by measuring shear stress as a function of shear rate from 0.1 to 100 $s^{-1}$.

### 2.6. Scanning electron microscopic (SEM) analysis of EPS

The lyophilised samples of EPS (10 mg $mL^{-1}$) were fixed to the SEM stubs with double sided tape. The samples were then coated with a layer of gold, ~10 nm thick. The samples were observed in a scanning electron microscope (FEI, Quanta 600 F, USA); accelerated voltage was operated at 15.0 kV.

### 2.7. Emulsifying properties of EPS

#### 2.7.1. Preparation of emulsions

The emulsifying activity of purified EPS of two strains of *Bifidobacterium* was compared with guar gum (plant origin) and xanthan gum (microbial origin) using the method of Cooper and Goldenberg (1987) and Freitas et al. (2011). Briefly, oil or xylene (3 mL) was added to 2 mL of EPS or gum solution (1%, w/v) in a screw cap glass tube (100 mm × 13 mm) and stirred in a vortex for 2 min at 40 Hz (Top mix FB 15024, Fisher Scientific, UK). After 24 h or 168 h, the emulsion index ($E_{24}$ or $E_{168}$) was determined as given below depending on the assay: $E_{24}$ or $E_{168} = (h_e/h_t) \times 100$, where $h_e$ is the height of the emulsion layer and $h_t$ is the overall height of the mixture. All samples were stored at 25 °C. All tests were performed in triplicate.

#### 2.7.2. Determination of emulsifying activity of EPS with different oils and xylene

Initially, different emulsions were prepared as described in Section 2.7.1 using EPS and gums with different oils (orange oil, sunflower seed oil, coconut oil and olive oil) and xylene as a hydrocarbon (control) to find the best oil which yields a stable emulsion based on $E_{24}$ and $E_{168}$. All chemicals used in the emulsion study were obtained from Sigma–Aldrich (UK).

#### 2.7.3. Effect of concentration of EPS on emulsion stability

Sunflower seed oil was selected as the best oil resulting in stable emulsions for both EPS as indicated by $E_{24}$ and $E_{168}$ (see Section 3) and hence was used for subsequent assays. Therefore, the emulsions prepared with sunflower seed oil were studied with respect to different concentrations of EPS of *B. infantis* NCIMB 702205 and *B. longum* subsp. *infantis* CCUG 52486 (0.25–1.5%) using the emulsion index $E_{24}$.

#### 2.7.4. Light microscopy and particle size distribution of emulsions

The particle size distribution of emulsions (1% EPS or gum, w/v) was measured using a modification of the method of Portilla-Rivera, Torrado, Dominguez, and Moldes (2010) and Ashtaputre and Shah (1995). Briefly, a light microscope (Leica DM 2500M, Leica Microsystems Ltd, UK) was used to examine and photograph the emulsion after 24 h storage at 25 °C through a 10× objective lens. A volume of 60 μL of the emulsion was added to a cavity microscope slide and kept for 5 min to settle down, followed by observation under the light microscope. The size distribution of the oil droplets in a single focal plane was calculated from three digital images of each sample using ImageJ software (National Institutes of Health, USA); values for equivalent diameter were extracted and histograms plotted.

### 2.8. Statistical analysis

The data were analysed with one-way analysis of variance (ANOVA) with SAS, version 9.2 (SAS Institute Inc., Cary, NC, USA). The comparison between means was carried out using the Tukey significant difference test.

## 3. Results and discussion

### 3.1. Physicochemical characteristics of EPS

As shown in Table 1, *B. infantis* NCIMB 702205 and *B. longum* subsp. *infantis* CCUG 52486 yielded around 241 and 366 mg $L^{-1}$ of EPS, respectively. However, *B. infantis* NCIMB 702205 and *B. longum* subsp. *infantis* CCUG 52486 produced 77 and 138 mg $L^{-1}$ of EPS, respectively, when they were grown in skim milk supplemented with 0.5% yeast extract under the same growing conditions (Prasanna et al., 2012b). This difference is due to the effect of protein source, as 1.5% of casein hydrolysate was used in this study, which was found to be the best dairy based protein source and concentration for EPS production by these two strains (Prasanna et al., 2012a). The molecular weight of EPS of *B. longum* subsp. *infantis* CCUG 52486 ($1.36 \times 10^6$ Da) was seventeen times higher than that of EPS of *B. infantis* NCIMB 702205 ($7.48 \times 10^4$ Da). These values were similar to the values that were reported previously for the same strains (Prasanna et al., 2012b). Both EPS were heteropolysaccharides composed of glucose and galactose and their composition were very similar to our previous study (Prasanna et al., 2012b).

The chemical composition of EPS depended on the *Bifidobacterium* strain (Table 1). Protein was not detected in either EPS, which indicated the effectiveness of the extraction and purification procedures adopted in this study, and resulted in high quality EPS. In contrast, higher protein content has been reported in some studies. In one study, *Bifidobacterium animalis* subsp. *lactis* IPLA-R1 was reported to produce EPS, which upon separation had a protein content of 3.9% (Leivers et al., 2011). Furthermore, 4.2% protein content was reported for the EPS isolated from *Bifidobacterium animalis* RH (Xu, Shen, Ding, Gao, & Li, 2011). In addition, higher protein content has been reported with some other bacterial species; including 8% of protein content for the EPS of *Bacillus* sp. 450 (Kumar Ganesh et al., 2004) and 10% of protein content for the EPS of *Pseudomonas oleovorans* (Freitas et al., 2009). These differences may be due to the different techniques used in the isolation and purification of EPS. The lyophilised EPS of *B. infantis* NCIMB 702205 and *B. longum* subsp. *infantis* CCUG 52486 were found to contain around 7.6% and 6.1% moisture, respectively, although they were freeze dried. This likely reflects trapped water being present in lyophilised EPS.

### 3.2. Intrinsic viscosity

Individual polysaccharide should be well separated in infinitely dilute solutions of polysaccharide and able to move independently with minimum interaction with other polymers (Piermaria, de la Canal, & Abraham, 2008). The intrinsic viscosity ($\eta$) is a measure of the hydrodynamic volume occupied by a given polymer in a given solvent (Freitas et al., 2011). In addition, it is a measure of a polymer's contribution to the viscosity of a solution. The intrinsic viscosity of EPS of *B. longum* subsp. *infantis* CCUG 52486 (47.4 dL $g^{-1}$) was fourteen times higher than that of EPS of *B. infantis* NCIMB 702205 (3.2 dL $g^{-1}$) and approximately three times that of guar gum (13.7 dL $g^{-1}$) (Table 1). However, this intrinsic viscosity of EPS of *B. longum* subsp. *infantis* CCUG 52486 was lower than that of xanthan gum (54.1 dL $g^{-1}$). The low intrinsic viscosity of EPS of *B. infantis* NCIMB 702205 suggests that it has a poor thickening ability and higher concentration should be used if high viscosity is desired. On the other hand *B. longum* subsp. *infantis* CCUG 52486 had a longer chain length and higher molar mass and therefore higher thickening ability than the EPS of *B. infantis* NCIMB 702205. In addition, the chain rigidity of the polymer can affect the intrinsic viscosity (Freitas et al., 2011). A similar intrinsic viscosity value was reported for xanthan gum (55.2 dL $g^{-1}$) in water but it was lower for guar gum (8.5 dL $g^{-1}$) under the same conditions (Parikh & Madamwar, 2006). There is no reliable information regarding the intrinsic viscosity of EPS produced by strains of *Bifidobacterium*. However, there are published studies describing the intrinsic viscosity of EPS of different LAB in water including a strain of *Lactococcus lactis* subsp. *cremoris* (19.6 dL $g^{-1}$) (Yang, Huttunen, Staaf, Widmalm, & Heikki, 1999); *Lactococcus lactis* subsp. *cremoris* B40 (32.0 dL $g^{-1}$) (Tuinier, Zoon, Stuart, Fleer, & de Kruif, 1999) and *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 (4.5 dL $g^{-1}$) (Grobben et al., 1997).

**Table 1**
Yield, molecular weight, monosaccharide ratio and composition of EPS of two strains of *Bifidobacterium* and intrinsic viscosity of two EPS, guar gum and xanthan gum in deionised water.

| Type of gum or exopolysaccharide | Yield (mg $L^{-1}$) (mean ± SD) | Molecular weight (Da) (mean ± SD) | Monosaccharide ratio | | Composition (%) | | | Intrinsic viscosity (dL $g^{-1}$) (mean ± SD) |
|---|---|---|---|---|---|---|---|---|
| | | | Glucose | Galactose | Carbohydrate | Protein | Water | |
| EPS of NCIMB 702205 | 241.5 ± 6.2 | $7.48 \times 10^4 \pm 6.32 \times 10^3$ | 1 | 2.5 | 92.34 | ND | 7.64 | 3.2 ± 0.1 |
| EPS of CCUG 52486 | 366.6 ± 4.4 | $1.36 \times 10^6 \pm 7.43 \times 10^4$ | 1.3 | 1 | 93.78 | ND | 6.19 | 47.4 ± 0.9 |
| Guar gum | – | – | – | – | – | – | – | 13.7 ± 0.4 |
| Xanthan gum | – | – | – | – | – | – | – | 54.1 ± 1.6 |

ND, not detected; –, not determined in this study.

### 3.3. Rheological properties of purified EPS solution

As shown in Fig. 1, the two EPS, xanthan gum and guar gum clearly showed a pseudoplastic or shear thinning property in aqueous solutions. The highest viscosities and the prominent shear thinning properties were observed for xanthan gum, followed by the EPS of *B. longum* subsp. *infantis* CCUG 52486, guar gum and the EPS of *B. infantis* NCIMB 702205. The hydrodynamic forces generated during the shear breakdown of the structural units of EPS cause the shear thinning behaviour of EPS (Khattar et al., 2010). Nevertheless, from a food product development point of view, this pseudoplastic property of EPS is important to yield good sensory properties such as mouth feel and flavour release properties of foods (Moreno et al., 2000). Furthermore, this property is important for various processes involved in food processing, such as mixing, pouring and pumping where different operative shear rates are applied. In addition, it is reported that polysaccharides with pseudoplastic, non-Newtonian, shear thinning behaviour are suitable for manufacturing different food products including dairy products, cake, salad dressing, syrups and puddings (Jindal et al., 2011).

In addition, a relationship was observed between the molecular weight of the polysaccharide and the viscosity in which the higher molecular weight of the polymer leads to higher viscosity. Therefore, a higher viscosity was observed with xanthan gum (Mw $\sim 2 \times 10^6$ to $5 \times 10^7$ Da, Sigma–Aldrich) (Papagianni et al., 2001) followed by the EPS of *B. longum* subsp. *infantis* CCUG 52486 (Mw $\sim 1.3 \times 10^6$ Da), guar gum (Mw $\sim 2.2 \times 10^5$ Da, Sigma–Aldrich) (Roberts, Elmore, Langley, & Bakker, 1996) and EPS of *B. infantis* NCIMB 702205 (Mw $\sim 7.4 \times 10^4$ Da). These results are in agreement with the observations of Muralidharan and Jayachandran (2003) who showed that the viscosity of the EPS solution is highly dependent on the average molecular weight. However, this is not in agreement with the explanation of De Vuyst et al. (2003) who reported that there is no clear-cut relationship between the molecular mass and the solution viscosity of a polysaccharide.

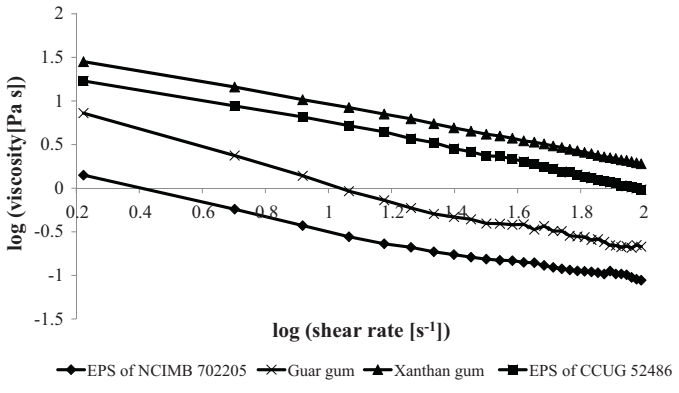


**Fig. 1.** Viscosity as a function of shear rate of aqueous solution of the two EPS, guar gum and xanthan gum, at concentrations of 1% (w/v).

### 3.4. Scanning electron microscopic analysis of EPS

Scanning electron microscopy is considered to be a powerful tool to study the surface morphology of macromolecules and could be used to elucidate their physical properties (Wang et al., 2010). A three dimensional and porous web like structure was observed with the EPS of *B. longum* subsp. *infantis* CCUG 52486 whereas the EPS of *B. infantis* NCIMB 702205 had a looser structure with relatively thinner and fragmented filaments (Fig. 2). Similarly, a porous structure was reported with purified EPS of *Leuconostoc dextranicum* NRRL B-1146 (Majumder & Goyal, 2009) and *Bacillus* sp. I-450 (Kumar Ganesh et al., 2004). At higher magnification (Fig. 2b and d) additional details of the microstructure of the two EPS are visible. Both EPS had smoother filaments rather than a sheet-like structure. There are no available SEM micrographs of EPS of *Bifidobacterium* strains to compare with our results. However, there are some SEM micrographs from a few other bacterial species. A sheet-like compact morphology of EPS was reported by Wang et al. (2010) for the EPS isolated from *Lactobacillus plantarum* KF5. In addition, the highly branched nature of the EPS of *B. longum* subsp. *infantis* CCUG 52486 compared to that of the EPS of *B. infantis* NCIMB 702205 could be the reason for the more viscous solution which was observed in this study. Moreover, the porous or web like structure of the EPS of *B. longum* subsp. *infantis* CCUG 52486 could result in a higher water holding capacity, which is a desirable characteristic for a texturing agent in the food industry.

### 3.5. Emulsifying activity

Microbial polysaccharides, plant gums and animal proteins, including xanthan gum, guar gum, whey protein concentrate and whey protein isolate, have been shown to have good emulsifying properties (Kanmani et al., 2011; Wang et al., 2008).

#### 3.5.1. Emulsifying stability of EPS of bifidobacteria with different oils and xylene

Emulsions prepared with the two EPS, xanthan gum and guar gum at a concentration of 1 mg $mL^{-1}$ using different oils and xylene were compared, and the results are given in Table 2. Both EPS showed a higher emulsification activity with sunflower seed oil, orange oil, coconut oil and xylene compared to xanthan and guar gum. There is no reported literature about the emulsification activity of the EPS produced by *Bifidobacterium* species. However, similar observations were reported with biopolymers produced by other bacterial species. More specifically, an emulsion prepared using hexadecane and the EPS of *Streptococcus phocae* PI80 was shown to have a higher emulsification activity (81.8) than guar gum (68.8) at 0.5 mg $mL^{-1}$ concentration (Kanmani et al., 2011). In another study, the EPS of *Lactobacillus kefiranofaciens* ZW3 was shown to have a higher emulsifying activity (88.0) with hexadecane compared to guar gum (37.4) at similar concentrations (Wang et al., 2008).

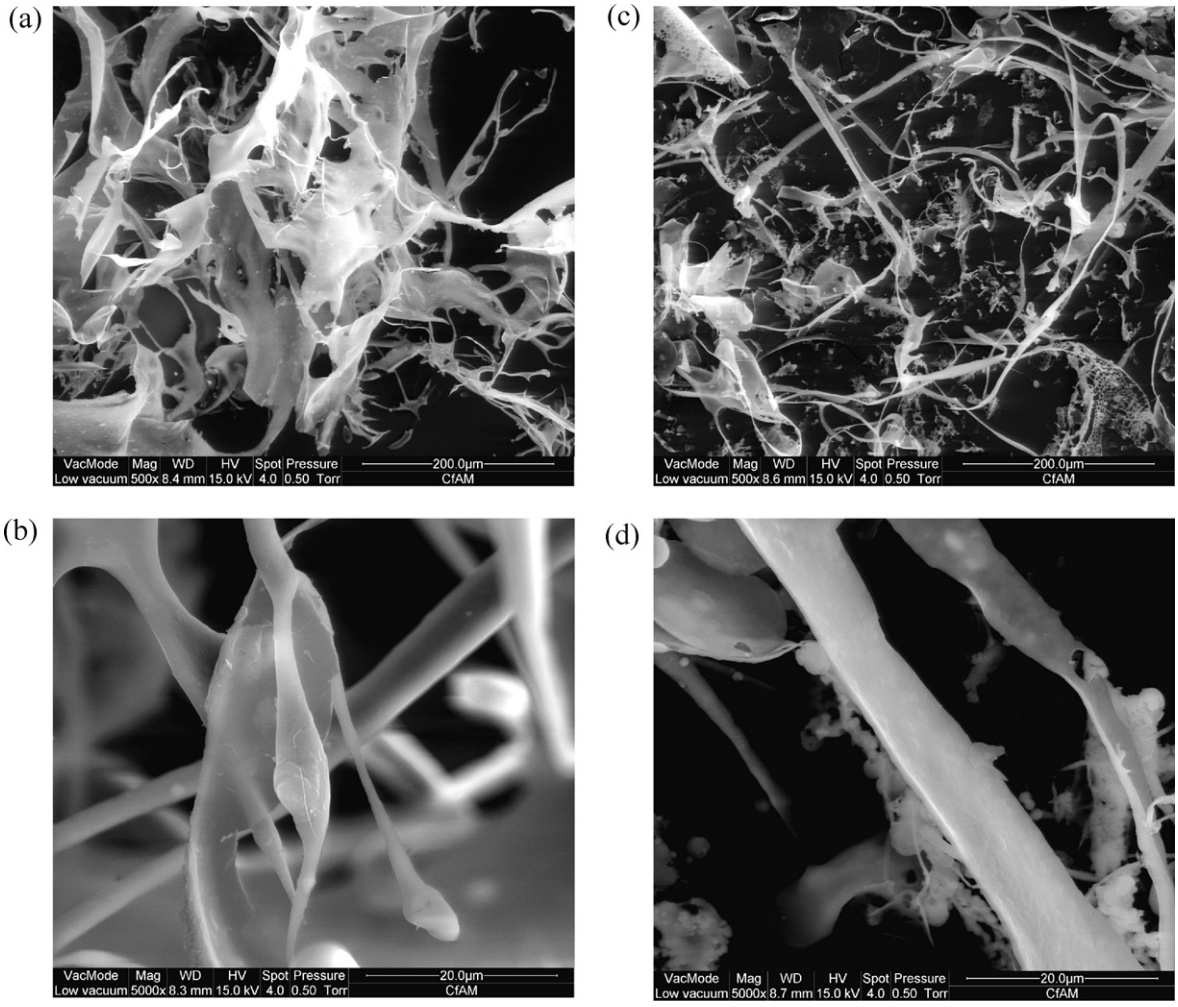


**Fig. 2.** Scanning electron micrograph showing the microstructure and surface morphology of purified EPS of *B. longum* subsp. *infantis* CCUG 52486 (a: 500×; b: 5000×) and EPS of *B. infantis* NCIMB 702205 (c: 500×; d: 5000×).

**Table 2**
Emulsification activity of EPS of *Bifidobacterium*, guar gum and xanthan gum (1 mg mL$^{-1}$) with xylene and different edible oils.

| Hydrocarbon or oil | Type of gum or exopolysaccharide | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Guar gum | | Xanthan gum | | EPS of NCIMB 702205 | | EPS of CCUG 52486 | |
| | $E_{24}$ | $E_{168}$ | $E_{24}$ | $E_{168}$ | $E_{24}$ | $E_{168}$ | $E_{24}$ | $E_{168}$ |
| Xylene | $51.4 \pm 2.8^{c}$ | $49.5 \pm 1.5^{c}$ | $53.3 \pm 1.6^{c}$ | $51.4 \pm 2.7^{c}$ | $59.0 \pm 1.6^{c}$ | $55.2 \pm 3.2^{c}$ | $60.9 \pm 1.6^{bc}$ | $53.3 \pm 4.3^{c}$ |
| Orange oil | $58.1 \pm 1.6^{bc}$ | $57.1 \pm 2.8^{abc}$ | $60.0 \pm 2.8^{bc}$ | $59.0 \pm 3.3^{b}$ | $62.8 \pm 2.8^{bc}$ | $60.9 \pm 1.6^{bc}$ | $60.1 \pm 4.3^{bc}$ | $60.0 \pm 5.7^{bc}$ |
| Sunflower seed oil | $65.7 \pm 2.6^{a}$ | $64.7 \pm 4.1^{a}$ | $69.5 \pm 1.6^{a}$ | $66.6 \pm 1.6^{a}$ | $78.2 \pm 4.0^{a}$ | $77.4 \pm 2.8^{a}$ | $72.2 \pm 2.8^{a}$ | $70.3 \pm 1.7^{a}$ |
| Coconut oil | $63.8 \pm 3.3^{ab}$ | $62.8 \pm 2.9^{ab}$ | $62.8 \pm 3.0^{ab}$ | $60.9 \pm 1.6^{ab}$ | $71.4 \pm 2.1^{ab}$ | $69.5 \pm 4.3^{ab}$ | $68.5 \pm 2.0^{ab}$ | $67.6 \pm 1.6^{ab}$ |
| Olive oil | $60.0 \pm 2.0^{ab}$ | $56.1 \pm 1.7^{bc}$ | $59.0 \pm 3.3^{bc}$ | $54.2 \pm 1.2^{bc}$ | $61.9 \pm 4.2^{c}$ | $57.1 \pm 2.5^{c}$ | $58.1 \pm 1.6^{c}$ | $52.3 \pm 1.5^{c}$ |

Mean values (±standard deviation) within the same column not sharing a common superscript differ significantly ($P < 0.05$). $E_{24}$, emulsification index after 24 h; $E_{168}$, emulsification index after 168 h.

The EPS of *B. infantis* NCIMB 702205 and *B. longum* subsp. *infantis* CCUG 52486 resulted in significantly higher emulsification stability with sunflower seed oil compared to the other edible oils except coconut oil. Furthermore, these emulsions prepared with sunflower seed oil and EPS were stable for 7 days at 25 °C. Similarly, higher emulsification stability with sunflower seed oil by some other bacterial EPS has been reported. More specifically, it was reported that the EPS of *Bacillus coagulans* RK-02 showed a significantly higher emulsifying activity with sunflower seed oil (70) than mustard oil (62), soybean oil (62), castor oil (50) and rice oil (41) (Kodali, Das, & Sen, 2009). According to Freitas et al. (2009) emulsion forming and stabilising capacity of EPS is specific for certain hydrophobic compounds. This may be the reason for the differences observed in this study.

### 3.5.2. *Effect of EPS concentration on emulsion stability*

In order to evaluate the possible effects of the EPS concentration on the emulsion stability, a number of studies were carried out using emulsions prepared with different concentrations of EPS and sunflower seed oil, and the emulsification indexes ($E_{24}$) were determined. Significant ($P < 0.05$) differences in the emulsification index were observed for the EPS of both strains when the emulsion was

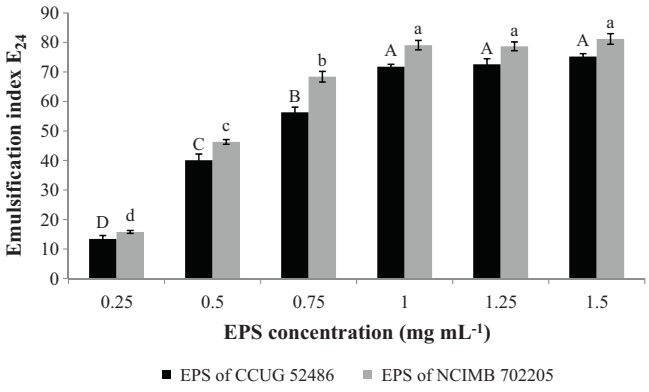


**Fig. 3.** Effect of different concentrations of the EPS of *B. infantis* NCIMB 702205 and the EPS of *B. longum* subsp. *infantis* CCUG 52486 on the emulsification index of the emulsions prepared with sunflower seed oil. The means for each organism with the same letters are not significantly different ($P < 0.05$). Vertical lines represent standard deviations. $E_{24}$: emulsification index after 24 h.

prepared with EPS concentrations ranging from 0.25 to 1 mg mL$^{-1}$ (Fig. 3). However, no significant differences ($P > 0.05$) in the emulsification index were observed when the concentration of EPS ranged from 1 to 1.5 mg mL$^{-1}$, although there was a small increase of the emulsion activity at higher concentrations. Therefore, 1 mg mL$^{-1}$ EPS concentration is the optimum concentration since there is no significant improvement of the emulsification activity at higher concentrations. Similar results have been obtained with the EPS of other bacterial species. Ashtaputre and Shah (1995) reported a similar trend in the emulsification index of the emulsions prepared with different concentrations of EPS (0.25–3 mg mL$^{-1}$) derived from *Sphingomonas paucimobilis* and xylene; they concluded that 1 mg mL$^{-1}$ was the best concentration for the emulsion. In another study, it was reported that 1 mg mL$^{-1}$ of EPS of *Enterobacter cloaceae* could effectively produce an emulsion with ground nut oil (Iyer et al., 2006).

### 3.5.3. Light micrographs and particle size distribution of emulsions

Fig. 4 shows typical micrographs for the emulsions prepared with the two EPS, guar gum and xanthan gum. The emulsion prepared with the EPS of *B. infantis* NCIMB 702205 was significantly different from the other emulsions. Smaller, densely packed and evenly distributed droplets were observed in the emulsion prepared with the EPS of *B. infantis* NCIMB 702205, whereas somewhat larger droplets were observed in the emulsion prepared of *B. longum* subsp. *infantis* CCUG 52486. Furthermore, large size droplets and flocculation of some droplets were observed in the emulsions prepared with guar gum and xanthan gum, whereas no flocculation was observed in the emulsion made with the EPS of *B. infantis* NCIMB 702205. However, the occurrence of flocculation or coalescence was prominent in the emulsion prepared with xanthan gum.

The emulsion droplet size is considered to be a very important parameter that determines the physical stability, such as flocculation and creaming rate of different emulsions (Kim, Morr, & Schenz, 1996). All the emulsions showed a monomodal distribution of droplets (Fig. 5). The emulsion prepared with the EPS from *B. infantis* NCIMB 702205 had significantly smaller droplet size, where more than 72% of particles were less than 5 μm and the emulsion droplets ranged from 1 to 36 μm. On the other hand, there was little variation in the particle size distribution of the emulsions prepared with the EPS of *B. longum* subsp. *infantis* CCUG 52486 (1–48 μm), guar gum (1–43 μm) and xanthan gum (1–45 μm). In addition, around 59% of droplets of the emulsion prepared with the EPS of *B. longum* subsp. *infantis* CCUG 52486, 60% of droplets with

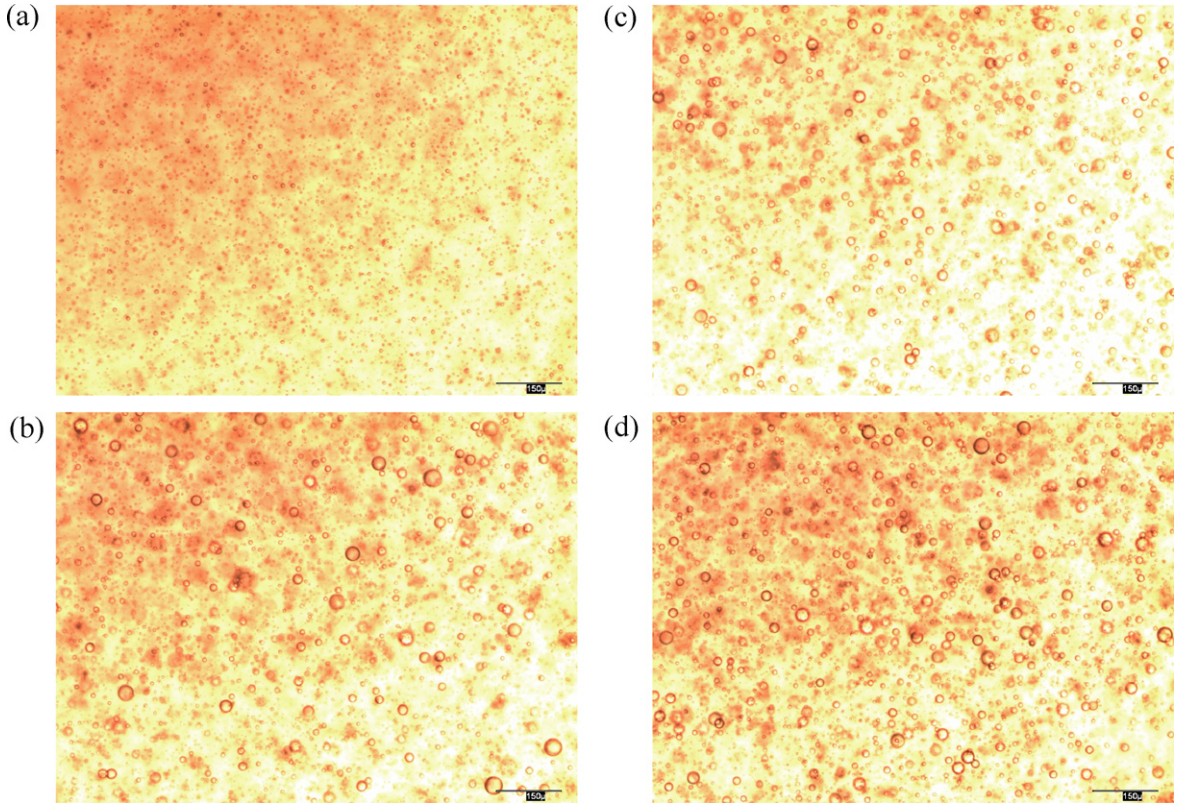


**Fig. 4.** Photomicrographs of emulsions prepared with 1% (w/v) of: (a) EPS of *B. infantis* NCIMB 702205, (b) EPS of *B. longum* subsp. *infantis* CCUG 52486, (c) guar gum and (d) xanthan gum (20×) with sunflower seed oil. The width of the bar corresponds to 150 μm.

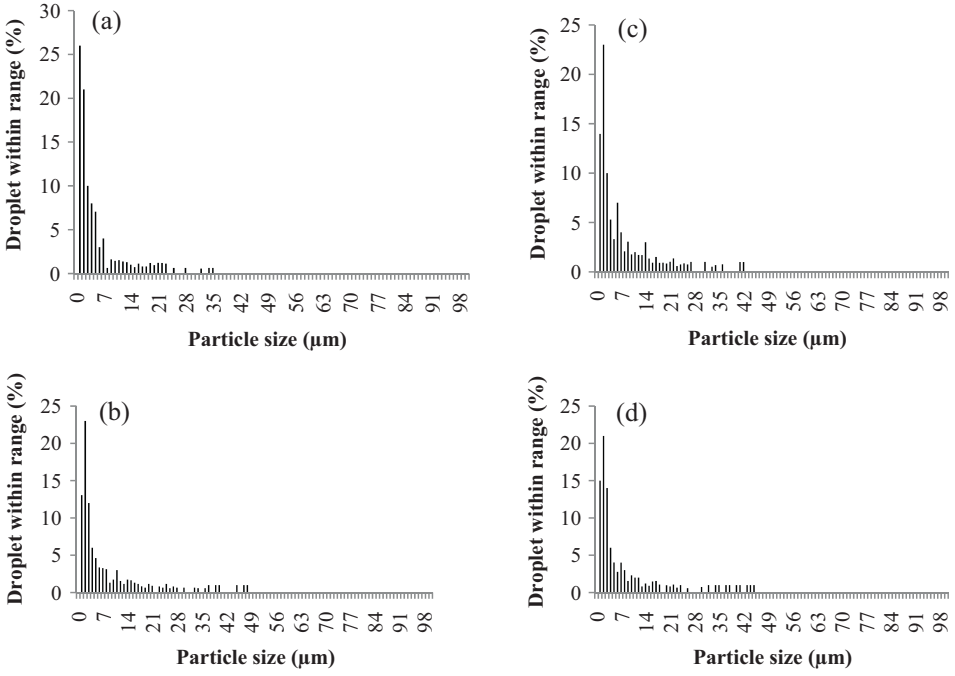


**Fig. 5.** Particle size distribution of emulsions prepared with 1% (w/v) of: (a) exopolysaccharide of *B. infantis* NCIMB 702205, (b) exopolysaccharide of *B. longum* subsp. *infantis* CCUG 52486, (c) guar gum and (d) xanthan gum with sunflower seed oil.

guar gum, and 56% of droplets with xanthan gum, were smaller than 5 μm. There is no recorded data of the emulsions prepared with the EPS of *Bifidobacterium* species to compare with these results. However, a droplet range of 10–40 μm was reported for an emulsion prepared with extracellular emulsifiers from *Lactobacillus pentosus* (Portilla-Rivera et al., 2010). In addition, Ashtaputre and Shah (1995) reported that more than 80% of the droplets of an emulsion prepared with 1% of EPS of *Sphingomonas paucimobilis* were less than 5 μm. Furthermore, it is reported that smaller droplets can result in more stable emulsions (Horozov, Binks, & Gottschalk-Gaudig, 2007). Therefore, these results reveal that the EPS produced by *B. infantis* NCIMB 702205 and *B. longum* subsp. *infantis* CCUG 52486 have great potential to be used as emulsifiers in the food industry.

## 4. Conclusions

*B. longum* subsp. *infantis* CCUG 52486 and *B. infantis* NCIMB 702205 produced heteropolysaccharides when they were grown in skim milk supplemented with casein hydrolysate. The EPS of *B. longum* subsp. *infantis* CCUG 52486 had a higher intrinsic viscosity value than the EPS of *B. infantis* NCIMB 702205 and guar gum. Both polymers showed non-Newtonian pseudoplastic behaviour. The SEM images revealed that the EPS of *B. longum* subsp. *infantis* CCUG 52486 had a three dimensional porous structure. The EPS of both *Bifidobacterium* strains stabilised the emulsions more effectively with different edible oils and xylene, compared to guar and xanthan gum. Stable emulsions were produced by both EPS at an optimum concentration of 1 mg mL$^{-1}$ with sunflower seed oil. Among the two EPS producing strains, the most promising one from an application point of view in the food industry is *B. longum* subsp. *infantis* CCUG 52486, as it produced considerable amount of EPS, together with a higher intrinsic viscosity and apparent viscosity in aqueous solution, and a porous dense entangled structure with higher emulsification activity, despite the fact that the EPS of *B. infantis* NCIMB 702205 had a slightly higher emulsification activity and smaller emulsion droplets.

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